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Effects of 11-ketotestosterone and fishmeal in the feed on growth of juvenile tilapia (*Oreochromis mossambicus*)

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ABSTRACT

The effects of 11-ketotestosterone (11KT) and dietary fishmeal on growth of tilapia (*Oreochromis mossambicus*) were examined. Juvenile tilapia, weighing about 8 g, were reared for 50 days with isonitrogenous and isocaloric diets containing fishmeal or all plant proteins, each with or without supplementation of 11KT. Fish fed fishmeal-based (FM) diets grew significantly faster than those fed soybean meal-based (SM) diets. Addition of 11KT (10 mg/kg) to the FM diet significantly augmented growth, whereas no significant effect of 11KT was observed in fish fed SM diet. In accord with the accelerated growth, specific growth rates of fish fed FM diets were significantly greater than those of fish fed SM diets, although addition of 11KT to FM or SM diet had no effect on this parameter. Males showed significantly higher plasma levels of insulin-like growth factor-I (IGF-I) than did females; however, there was no consistent effect of fishmeal or 11KT on plasma IGF-I levels within each sex. Plasma levels of 11KT were significantly higher in males than in females. In males, plasma 11KT levels were significantly higher in fish fed FM diets than in those fed SM diet. No significant effect of fishmeal or 11KT was observed on plasma levels of 11KT or 17β-estradiol (E_2) in females. Low but significant levels of vitellogenin (Vg) were found in male plasma. Plasma Vg levels were significantly lower in fish fed SM diets than in fish fed FM diets in both males and females. These results indicate that addition of 11KT to the fishmeal-based diet stimulated growth, minimally affecting plasma levels of IGF-I, 11KT, E_2 and Vg. The absence of effect of 11KT in fish fed the soybean meal-based diet may indicate that the estrogenic activities or some components in the SM diet are likely to interfere with the growth-promoting effects of 11KT.

1. Introduction

Tilapias have become a major source of protein around the world, primarily because of their outstanding adaptability under a wide range of environmental conditions and excellent growth on a variety of natural and prepared diets (Lim and Webster, 2006). Farming carnivorous and omnivorous fish species, such as salmonids, catfish and tilapia, puts an additional demand on ocean fisheries, since wild species remain the principal ingredient of their feed (Pauly et al., 2005; Tacon and Metien, 2008). Attempts have been made to replace fishmeal in tilapia diets with various plant proteins, such as cottonseed, sunflower, soybean, corn, and broad bean (El-Saidy and Gaber, 2002, 2003; Gaber, 2006; Jackson et al., 1982; Mbahinzireki et al., 2001; Ofojekwa and Ejike, 2003). In some cases, however, the complete replacement of fishmeal in feed with plant protein led to lower growth compared with fish fed fishmeal-containing diets.

We have been utilizing euryhaline Mozambique tilapia (*Oreochromis mossambicus*) as a model species for the study of growth physiology of fish in fresh water and also in seawater (Shepherd et al., 2006). Males of this species grow faster than females. We have shown that the difference in growth between male and female tilapia is likely to be due to complex interactions between the growth hormone (GH) and insulin-like growth factor-I (IGF-I) axis and gonadal steroid hormones (Riley et al., 2004; Shepherd et al., 2006). For example, addition of 17α-methyltestosterone (MT) to the feed during early development of Mozambique tilapia produced populations with a high frequency of males that grow faster than untreated, mixed-sex control animals (Howerton et al., 1992; Kuwaye et al., 1993; Riley et al., 2002b; Ron et al., 1995; Sparks et al., 2003). By contrast, similar treatment of tilapia with a synthetic estrogen, ethynylestradiol (EE_2), produced a high proportion of females, which grew more slowly than the controls (Meredith et al., 1999). According to Riley et al. (2002a), mature male tilapia treated with 17β-estradiol (E_2) significantly elevated plasma levels of GH, while reducing plasma IGF-I. By contrast, females treated with dihydrotestosteone (DHT) showed lower plasma GH with elevated plasma IGF-I. Available evidence suggests that female tilapia may direct energy from

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somatic growth toward egg production, specifically toward the production of vitellogenin (Vg), a major yolk precursor protein (Riley et al., 2004).

In fish, testosterone serves as a natural precursor for other sex steroids such as 11-ketotestosterone (11KT) in males and E_2 in females, which play important physiological roles during testicular and ovarian development, respectively (Ijiri et al., 2008; Schulz et al., 2001). Feeding of 11KT increased the food conversion efficiency in juvenile carp (Lone and Matty, 1982). Toguyeni et al. (1997) observed that juvenile male Nile tilapia (*O. niloticus*) displayed higher specific growth rate and lower feed conversion ratio than females, and suggested that higher plasma levels of 11KT may be involved in the higher metabolic capacity of males. According to Larsen et al. (2004), injection of immature salmon with 11KT produced an increase in plasma IGF-I levels after 1 and 2 weeks. Nonetheless, there seems to be no report on long-term growth-promoting effects of 11KT in tilapia, or in any other teleost species. The present study attempted to determine whether the 11KT added to isonitrogenous and isocaloric diets, with or without fishmeal, possesses a growth-promoting effect in juvenile Mozambique tilapia.

2. Materials and methods

2.1. Rearing

Juvenile tilapia (*O. mossambicus*) were obtained from brood stock at the Hawaii Institute of Marine Biology, University of Hawaii. They were maintained in 700 L freshwater flow-through tanks under natural photoperiod and were fed approximately 2% of the body weight per day with Silver Cup Trout Chow (Nelson and Sons, Murray, UT). Experiments were conducted in accordance with the principles and procedures approved by the Institutional Animal Care and Use Committee, University of Hawaii.

2.2. Experimental feeds and feeding

Two isonitrogenous (32% protein) and isocaloric diets (2900 kcal/kg), a fishmeal-based (FM) diet and a soybean meal-based (SM) diet, were processed into pellets, dried at room temperature and stored frozen at −20 °C as described by Perez et al. (2003). The composition and estimated nutrient content of the experimental feeds are shown in Table 1. 11-ketotestosterone (11KT, Sigma, St. Louis, MO) was added to the pellets by spraying 1 kg of feed with 10 mg of 11KT dissolved in 50 ml of 95% ethanol. The diet without 11KT was prepared by spraying 1 kg feed with 50 ml of 95% ethanol. Following evaporation of ethanol in air, the feed was stored at −20 °C. We have estimated the content of 11KT in the feed by specific radioimmunoassay as described below, and confirmed that 11KT is evenly distributed in the feed (10.66 ± 0.71 mg/kg, $n = 6$).

Seven hundred and twenty fish, weighing approximately 8 g, were tagged individually with passive integrated transponder tags (PIT tags; Biomark, Boise, ID) and divided randomly into 12 tanks representing three replicates of 4 treatment groups. These included: FM diet with (FM+) or without (FM−) 11KT; and SM diet with (SM+) or without (SM−) 11KT. The animals were maintained outdoors in oval 700 L fiberglass aquaria (60 fish/tank). Water temperature was maintained at 26 ± 2 °C using submersible aquarium heaters (Ebo-Jager Inc., El Segundo, CA). The fish were allowed to acclimate to the tanks for 2 weeks prior to the beginning of the experiment. During this period, fish were fed a maintenance diet of trout chow (Nelson & Sons, Inc., Murray, UT) at approximately 2% body weight at each of two feedings; at 8 AM and 3 PM. After confirming that all fish gained weight at similar rates during the 2 weeks of acclimation, fish in 3 tanks, chosen at random, were fed each of the 4 experimental diets (FM+, FM−, SM+, SM−), twice daily (2% body weight per feeding) at 8 AM and 3 PM to apparent satiation by feeding each tank of fish as much food as they could eat in 30 min. Only a small amount of the food was left in the tanks, which was then carefully removed by a net. The amounts of the feed consumed for each tank were estimated daily by calculating the differences between the initial dry weights of the feed prepared for each tank and the weight of the remaining feed after feeding.

Table 1
Composition of experimental diets with and without fishmeal.

Ingredients	Percent in diet	
	With fishmeal	Without fishmeal
Fish meal (menhaden)	25.0	–
Soybean meal	21.0	46.4
Cottonseed meal	–	12.0
Wheat middlings	16.0	16.0
Corn meal	32.5	16.0
Carboxy-methyl cellulose	3.0	3.0
Dicalcium phosphate	–	1.5
Soybean oil	1.5	3.9
DL-methionine	–	0.2
Vitamin mix[a]	0.5	0.5
Trace mineral mix[b]	0.5	0.5
Estimated nutrients (at 90% DM)		
Crude protein (% as is)	32.0	32.0
Crude fat (%)	6.0	6.0
Digestible energy (kcal/diet)	2930	2910

[a] Vitamin mix, diluted in cellulose, provides the following in mg/kg diet: vitamin A (500,000 IU/g), 12, vitamin D3, (1,000,000 IU/g), 2; vitamin K (51%), 20; vitamin E (50%), 200; thiamin, 15; riboflavin, 30; pyridoxine, 20; panthothenate, 200, nicotinic acid, 150; flolic acid, 2; vitamin B12 (0.1%), 2; biotin, 1; inositol, 200; choline chloride, 1000; Stay C (50%, Na-ascorbyl-phosphate), 300.

[b] Mineral mix, diluted in cellulose, provides the following in mg/kg diet: Zinc (as $ZnSO4 \cdot 7H_2O$), 150; ion (as $FeSO4 \cdot 7H_2O$), 25; copper (as $CuCl_2$), 3; iodine (as KI), 5; cobalt (as $CoCl_2 \cdot 6H_2O$), 0.05; selenium (as $NaSeO_2$), 0.1.

2.3. Sampling

Ten fish from each of the 12 tanks were sampled and sacrificed at days 0, 10, 20, 30, 40, and 50. Sampling started at 9 AM, or 25 h after the last feeding (no feed was given at the second feeding time of 3 PM the day before to ensure no feed remained in the stomach). To eliminate the effect of crowding stress on growth rate, stocking densities were maintained at approximately 5 g/L by adjusting the height of the vertical standpipe as fish were removed for sampling. Following each sampling, fish were closely observed for any indication of stress (e.g., color or behavioral changes). There was no indication of stress observed and all fish remaining in the tanks resumed eating in the afternoon (3 PM). The fish were sexed by visual observation of the genital papilla. Both males and females were at a stage of gonadal immaturity at the beginning of the experiment. At the end of the experiment (day 50), maturational stages were identified by eye; vitellogenic oocytes were observed in most females, and some ovulating females were found, while milt was observed in some of the larger males.

Individual body weight and standard length were measured during each sampling. Specific growth rate $(\ln W_f - \ln W_i)/t \times 100$, where W_f is the final weight (g), W_i is the initial weight (g) at each time interval and t is time (days), as well as condition factor, $(100 \times W/L^3)$, where W is body weight (g) and L is standard length (cm), were calculated at each time point. At the end of the experiment (day 50), all fish were anesthetized in buckets containing 2-phenoxyethanol (Sigma, 0.2 mL/L), and blood was collected from the caudal vasculature by a needle and syringe treated with ammonium heparin (200 U/mL, Sigma). Plasma was separated by centrifugation at $10{,}000 \times g$ for 10 min at 4 °C, and stored at −80 °C until analyses for plasma levels of IGF-I, 11KT, E_2, and Vg. Gonado somatic index (GSI) was not estimated in the present study primarily because at the first

several samplings, most of the fish, particularly males, were still immature with small gonadal size, even at the end of the experiment.

2.4. Intraperitoneal injection of 11KT

The time course of changes in plasma levels of 11KT was examined after intraperitoneal injection of 11KT (1 μg/g) into mature females (100–150 g). The dose of 11KT was equivalent to the amount of 11KT in the feed (10 mg/kg), assuming that fish consumed 10% of body weight at one time. Fish were anesthetized with 2-phenoxyethanol and injected with 11KT, dissolved in soybean oil (1 mg/ml). Serial blood samples from each fish were taken from caudal vessels at 0 (at the time of injection) and 4, 8, and 24 h post-injection. Plasma was collected by centrifugation at 10,000×*g* for 10 min and stored at −80 °C. In our previous study, repeated blood withdrawal had no significant effect on plasma cortisol levels, although there were significant increases in plasma prolactin levels (Leedom et al., 2003). Unlike some salmonid species, tilapia seems to be less susceptible to handling stress and changes in blood volume.

2.5. Measurements of plasma hormones

Plasma levels of IGF-I, Vg, 11KT, and E_2 were estimated in samples taken 24 h post-feeding at the end of the experiment (Day 50). Total IGF-I levels were measured using recombinant salmon IGF-I as a standard and anti-barramundi IGF-I (GroPep, Adelaide, Australia) following Shimizu et al. (1999) and Kajimura et al. (2001). Plasma Vg levels were estimated by an enzyme-linked immunosorbent assay (ELISA) based on Denslow et al. (1999) and modified by Davis et al. (2007).

Plasma levels of 11KT were estimated using an 11KT standard (Sigma), 11KT antibody (anti-11-oxo-testosterone, Cosmo FKA 118, Cosmo Bio, Tokyo) and ^{3}H-11KT (Amersham, Piscataway, NJ). Plasma 11KT was extracted with ether as follows: 2 ml of ethyl ether (Sigma) was added to 50–200 μl of plasma diluted 1:2 in distilled water; the samples were vortexed, kept frozen at −80 °C for 10 min, and the aqueous organic layer was decanted. The ether extract was evaporated to dryness in a water bath at 40 °C for 1 h and then placed under nitrogen for 5 min to ensure complete evaporation. Extracts were then reconstituted with assay buffer (0.01 M PBS and 1% BSA; all reagents from Sigma). The 11KT standards were prepared by diluting 11KT to desired concentrations with assay buffer; 100 μl of assay buffer containing standard was added to the assay tube in duplicate. All tubes, except those for total and non-specific binding, received 100 μl anti-11KT antibody at 1:15,000 dilution in assay buffer. Tubes were incubated for 2 h at room temperature prior to addition of 100 μl ^{3}H-11KT and then incubated overnight at 4 °C. Following incubation, 400 μl dextran-coated charcoal (0.5% charcoal, 0.05% dextran, 0.01 M PBS, Sigma) was added to all tubes except those for the total binding. Tubes were incubated on ice for 20 min, centrifuged at 1000×*g* for 20 min and the supernatant was counted in a beta counter (LS3801, Beckman Coulter, Fullerton, CA). Validity of the assay was assessed from the parallel displacement curves obtained with serial dilutions of plasma samples and the absence of cross-reaction in charcoal stripped plasma. Plasma samples were stripped by incubation for 15 min with 2% w/v activated charcoal (Sigma) at room temperature followed by centrifugation at 10,000×*g* for 5 min, and supernatant collected as stripped extract. Intra- and inter-assay coefficients of variation were 1.0% ($n=10$) and 2.6% ($n=9$), respectively.

Plasma levels of E_2 were estimated essentially following a protocol that was similar to that used for 11KT, using anti-E_2 antibody (#244, anti-E_2-6-BSA, Colorado State University at a concentration of 1:80,000) and ^{3}H-E_2 (Amersham, Piscataway, NJ). Intra- and inter-assay coefficients of variation were 9.8% ($n=5$) and 19.1% ($n=5$), respectively.

2.6. Statistical analyses

Statistical analyses were conducted using one-way or two-way analysis of variance (ANOVA) with treatment and time as independent variables followed by Fisher's least significant difference test. Repeated measures analysis followed by paired *t*-test was used for time course of changes in plasma 11KT after intraperitoneal injection. All analyses were conducted using a computer program, STATISTICA (StatSoft, Tulsa, OK). Significant level was set at $P<0.05$. Data are expressed as means ± SEM.

3. Results

The effects of 11KT and fishmeal in the feed on mean growth rate and feed consumption are shown in Fig. 1. Significant main effects of treatments ($P<0.01$) and time ($P<0.001$) were observed on growth rate. Overall, fish fed FM diets grew significantly ($P<0.001$) greater than those fed SM diets. Addition of 11KT (10 mg/kg) to the FM diet (FM+) significantly ($P<0.01$) augmented growth, while no significant effect of 11KT was observed in the fish fed SM diet. On days 40 and 50, fish fed the FM diet without 11KT (FM−) were significantly larger than the fish fed the SM diet without 11KT (SM−). When

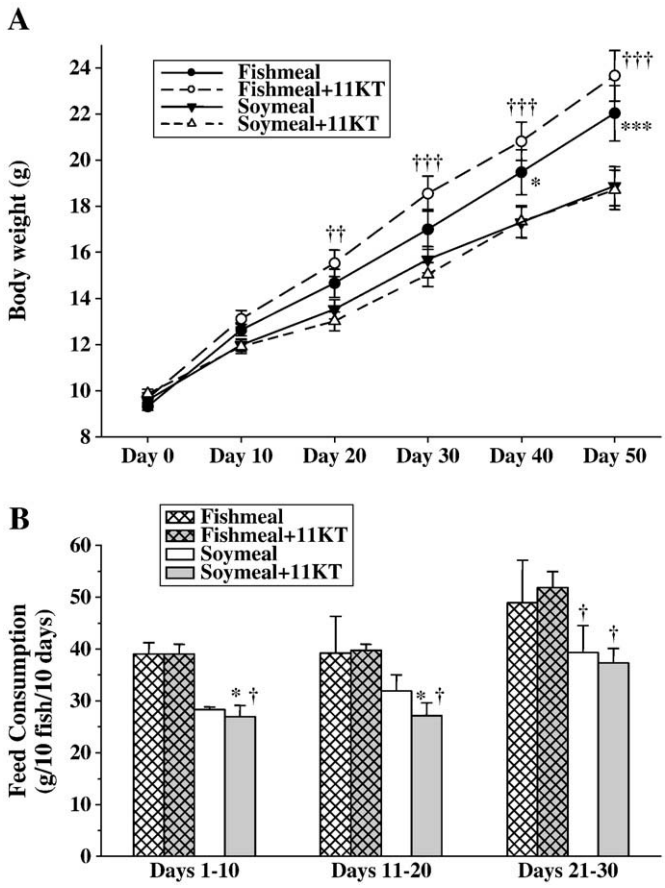

Fig. 1. Effects of 11KT and fishmeal in the feed on growth rate (A) and feed consumption (B). Mean ± SEM ($n=27$–30 for growth, and $n=3$ for feed consumption). Significant main effects of treatments ($P<0.01$) and time ($P<0.001$) were observed in growth. There were significant main effects of treatments ($P<0.001$) and time ($P<0.01$) in feed consumption. *, **, *** Significantly different from the fish fed soybean meal-based diet without 11KT (SM−) at corresponding period at $P<0.05$, 0.01 and 0.001, respectively. †, ††, †† Significantly different from the fish fed soybean meal-based with 11KT (SM+) at corresponding period at $P<0.05$, 0.01 and 0.001, respectively (A). For feed consumption (B), comparison was made with the fish fed fishmeal-based diet without11KT (FM−, *$P<0.05$) or with 11KT (FM+, †$P<0.05$) at corresponding period. Feed consumption for the fish fed fishmeal-based diet with 11KT was significantly greater during Days 21–30 compared with the initial level (Days 1–10, $P<0.05$).

compared with SM+ group, fish fed FM diet with 11KT (FM+) had significantly greater growth from day 20 to day 50 (Fig. 1A). There was no significant difference in growth rate between males and females of any treatment group (data not shown).

Feed consumption was estimated until day 30. Overall, fish fed SM diets consumed significantly ($P<0.01$) less feed than fish fed the FM diets. Compared with FM− or FM+ groups, a significant ($P<0.05$) reduction in feed consumption was observed in SM+ groups throughout the experiment. A significant reduction in feed consumption was also seen in the SM− group compared with the FM+ group during days 21–30 (Fig. 1B). Feed conversion ratio (feed consumed/weight gain) was variable among treatment groups and time, ranging from 1.2 to 2.4, and no significant effect of 11KT or fishmeal was observed (data not shown).

Fig. 2 shows the effects of 11KT and fishmeal in the feed on specific growth rate (A) and condition factor (B). There were significant ($P<0.001$) main effects of treatment and time on specific growth rate. Specific growth rates during the first 10 days were significantly ($P<0.001$) greater for all groups than throughout the rest of the experimental period. Specific growth rates of the FM− fed fish were significantly ($P<0.001$) greater than in those fed SM diets; no effect of 11KT was observed in the fish fed FM diet or SM diet (Fig. 2A).

Condition factor of the fish fed FM− diet was significantly ($P<0.01$) lower than those fed SM+ or SM− diets. In all experimental groups, condition factor during the first 10 days was significantly ($P<0.001$) greater than those during the rest of the experiment. No significant effect of 11KT was seen in the fish fed either the FM+ diet or the SM+ diet. Compared with the fish fed the FM− diet, significantly higher condition factors were observed in the fish fed the SM+ or SM− diets on days 30 and 40 (Fig. 2B). There was no significant sex-related difference in specific growth rate or condition factor (data not shown).

Plasma levels of IGF-I, 11KT, E_2 and Vg were estimated at the end of the experiment (day 50), when the fish reached 18–22 g and sufficient plasma samples (about 200 µl) were obtained for the measurements. Males showed significantly ($P<0.01$) higher levels of plasma IGF-I than in females. Compared with the fish fed FM− diet, significantly ($P<0.05$ or 0.01) higher levels of plasma IGF-I were observed in the males fed SM+ diet and in females fed FM+ and SM− diets. No significant effect of 11KT was seen in males fed SM diet (Fig. 3A).

Plasma levels of 11KT in males were significantly ($P<0.001$) higher than in females, and no significant effect of fishmeal or 11KT was observed in females. In males, plasma 11KT levels were significantly ($P<0.01$) lower in the fish fed SM+ or SM− diets than in those fed

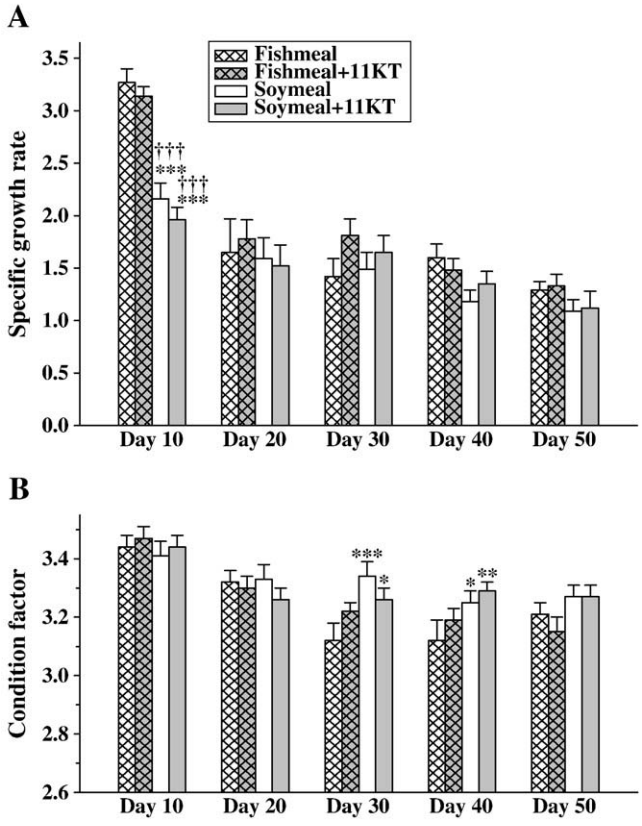

Fig. 2. Effects of 11KT and fishmeal in the feed on specific growth rate (A) and condition factor (B). Mean ± SEM ($n=27–30$). There were significant main effects of treatments ($P<0.001$) and time ($P<0.001$) on specific growth rate. There were also significant main effects of treatment ($P<0.05$) and time ($P<0.001$) on condition factor. *, **, *** Significantly different from the fish fed fishmeal-based diet without 11KT (FM−) at corresponding period at $P<0.05$, 0.01 and 0.001, respectively. ††† Significantly different from the fish fed fishmeal-based diet with 11KT (FM+) at corresponding period at $P<0.001$. All the values of specific growth rate for each treatment from Day 20 to Day 50 were significantly ($P<0.01$) lower than the corresponding level on Day 10, and all the values of condition factor for each treatment from Day 20 to Day 50 were significantly ($P<0.05$) lower than the corresponding level on Day 10, except for the fish fed FM− on Day 20 and the fish fed soybean meal-based without 11KT (SM−) on Days 20 and 30.

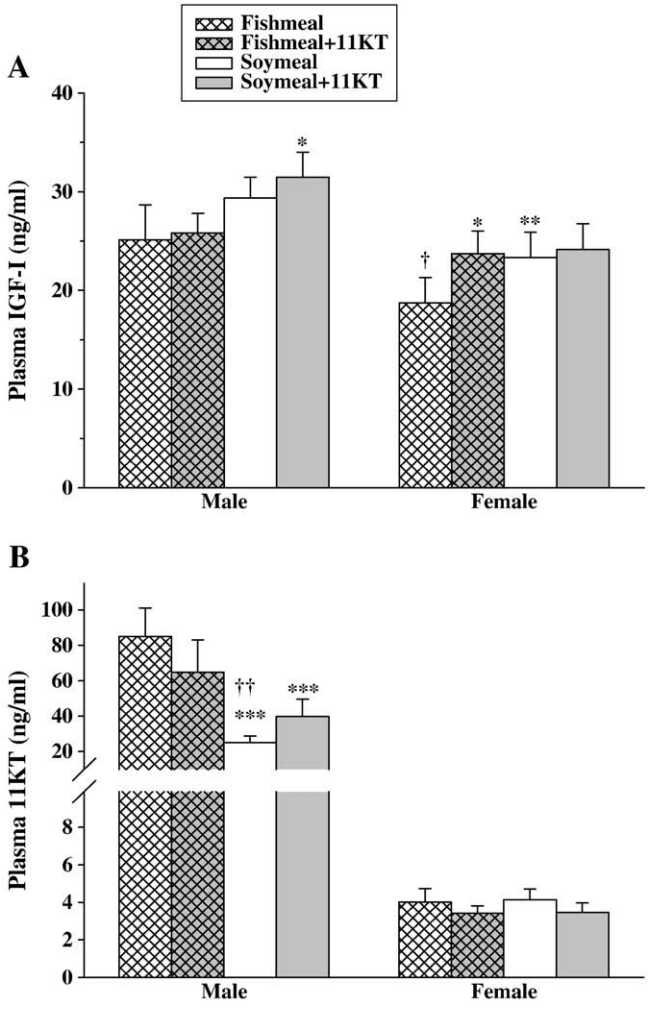

Fig. 3. Effects of 11KT and fishmeal in the feed on plasma levels of IGF-I (A) and 11KT (B). Mean ± SEM ($n=10–17$). Plasma samples were taken 24 h after the last meal at the end of the experiment (Day 50). There were significant ($P<0.01$) main effects of both sex and treatment on plasma IGF-I. There were also significant main effects of sex ($P<0.001$) and treatment ($P<0.05$) in plasma 11KT levels. *, ** Significantly different from the fish fed fishmeal-based diet without 11KT (FM−) for each sex at $P<0.05$, 0.01 and 0.001, respectively. †, †† Significantly different from the fish fed fishmeal-based diet with 11KT (FM+) for each sex at $P<0.05$, and 0.01, respectively.

FM+ or FM− diets. There was no significant effect of 11KT in males fed FM+ or SM+ diet (Fig. 3B). There was no significant correlation between body weight and plasma IGF-I or 11KT levels in either males or in females (data not shown).

Low but significant levels of Vg were found in male plasma (0.2–3 mg/ml). The fish fed FM diets had significantly higher levels of plasma Vg than those fed SM diets. The presence of 11KT in the FM diet significantly ($P<0.01$) increased the Vg levels, whereas 11KT was without effect in the SM diet. In females, plasma Vg levels (10–30 mg/ml) were significantly ($P<0.001$) higher than in males. As in the case of males, fishmeal in the diet significantly ($P<0.01$) increased plasma Vg in female tilapia. In contrast with males, however, females fed the FM+ diet had significantly ($P<0.05$) reduced Vg levels, whereas there was no effect of 11KT in fish fed the SM diet (Fig. 4A). No significant effect of 11KT or fishmeal was observed on plasma levels of E_2 in females (Fig. 4B). There was no significant correlation ($P=0.13$) between plasma levels of Vg and E_2 in females. Plasma E_2 levels were not examined in males due to paucity of the plasma samples.

Since there was no significant change in male or female plasma 11KT levels when 11KT was supplemented to FM or SM diets, the time course of changes in plasma 11KT were examined after intraperitoneal injection of 11KT (1 μg/g) into mature females. The dose of 11KT was equivalent to the amount of 11KT in the feed (10 mg/kg), if the fish consumed 10% of body weight at one time. As shown in Fig. 5, plasma levels of 11KT increased markedly to more than 200 ng/ml 4 h after the injection, decreased to approximately 30 ng/ml after 8 h, and returned to the initial level after 24 h.

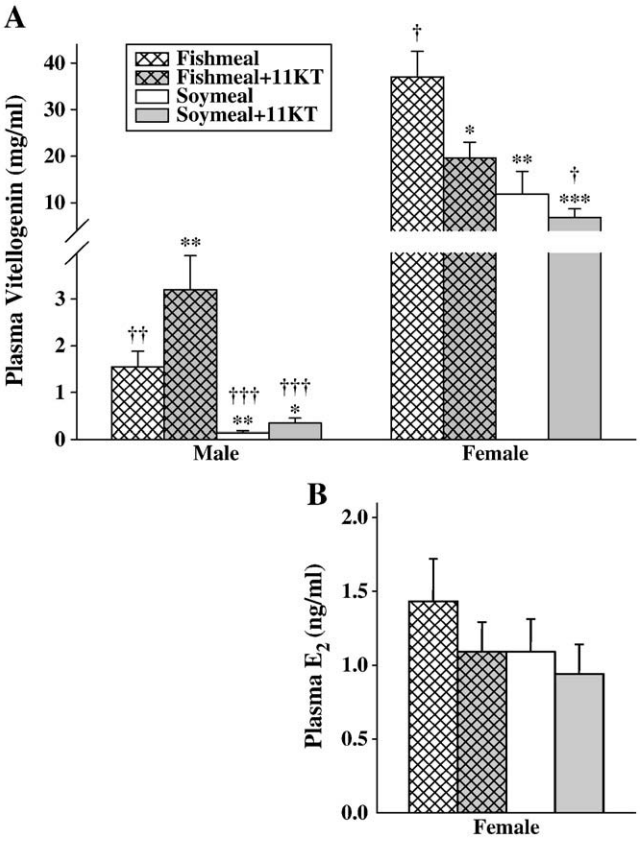

Fig. 4. Effects of 11KT and fishmeal in the feed on plasma levels of vitellogenin (A) and 17β− estradiol (E_2) (B). Plasma samples were taken 24 h after the last meal at the end of the experiment (Day 50). Results on males and females were analyzed separately by one-way ANOVA. *, **, *** Significantly different from the fish fed fishmeal-based diet without 11KT (FM−) for each sex at $P<0.05$, 0.01, and 0.001, respectively. †, ††, ††† Significantly different from the fish fed fishmeal-based diet with 11KT (FM+) for each sex at $P<0.05$, 0.01, and 0.001, respectively.

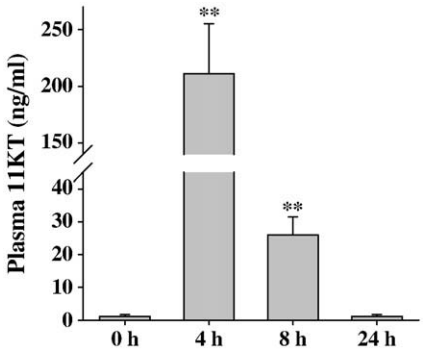

Fig. 5. Time course of changes in plasma 11KT after intraperitoneal injection of 11KT (1 μg/g) into mature females. Mean ± SEM ($n=6$). ** Significantly different from the initial level (time 0) at $P<0.01$.

4. Discussion

In the present study, 11KT, the native androgen in most teleost species, including the tilapia, was utilized to investigate the effect of androgens in the feed on growth physiology in tilapia. Although commercial use of this compound may not, at present, be viable, understanding the actions of any intrinsic hormone is important for future optimization of fish production and energy usage in aquaculture. The results of the present study clearly indicate that juvenile Mozambique tilapia fed FM diets for 50 days grew significantly faster than those fed SM diets, and that 11KT in the FM diet further accelerated the growth, whereas 11KT was without effect in the SM diet. In accord with the accelerated growth, specific growth rates in the fish fed FM diets were significantly greater than in those fed SM diets. The augmented growth by adding 11KT to FM diet (FM+) is in agreement with our previous findings using the same concentration of methyltestosterone (MT, 10 mg/kg) added to trout chow and given to tilapia from fry stages for 150–200 days (Howerton et al., 1992; Riley et al., 2002b; Ron et al., 1995; Sparks et al., 2003). Androgens have been found to stimulate growth by increasing feed intake or by improving feed conversion ratio in several teleost species (Donaldson et al., 1979). According to Toguyeni et al. (1997), higher plasma levels of 11KT in male tilapia may produce the higher metabolic capacity of the males compared with females and result in higher specific growth rate and lower feed conversion ratio. In the present study, the condition factor of the fish fed FM− diet was significantly lower than for those fed SM+ or SM− diets; however, 11KT or fishmeal was without a significant effect on feed conversion ratio (data not shown). It is not clear why specific growth rate and condition factor in all the groups were significantly greater for the first 10 days than the rest of the experimental period.

The feed consumption of fish fed SM diets was significantly lower than that of fish fed FM diets during the first 10 days. The reduced feed consumption in the fish fed SM diets may be due to a difference in palatability. It is generally recognized that palatability of all-plant diets, even formulated to contain similar essential nutrient levels, is poorer than that of diets containing fish meal. Commercial fish feeds for fry often contain fishmeal, which can comprise up to 65% of the diet. Fishmeal is prepared from whole fish and fish by-products and can contain high levels of sex steroid hormones (Pelissero and Sumpter, 1992). In addition, commercial fish feeds are also known to contain phytoestrogens (Ishibashi et al., 2002; Kobayashi et al., 2006). The sex steroids in the fishmeal or androgenic or estrogenic activities in the other components of the fish feed may potentiate the growth-

promoting effects of 11KT. Alternatively, the absence of effect of 11KT in the fish fed SM diets may indicate that estrogenic activities or some components in the SM diet potentially mask or interfere with the effect of 11KT on tilapia growth.

Long-term treatment of tilapia with MT starting from yolk-sac fry stage for 150–200 days produced populations with a high frequency of males that grow faster than untreated, mixed-sex control animals (Howerton et al., 1992; Kuwaye et al., 1993; Riley et al., 2002b; Ron et al., 1995; Sparks et al., 2003), whereas similar treatment with EE_2 produced a high proportion of females, which grew more slowly than controls (Meredith et al., 1999). According to Nakamura et al. (1998), gonadal differentiation in tilapia appears to occur as early as 8 days after hatching for females and after 35–40 days post-hatch for males. In the current study, 11KT was given to the fish weighing about 8 g, and 11KT did not appear to induce sex change in FM or SM groups. The fish used for this study were at a stage of gonadal immaturity at the beginning of the experiment. Nonetheless, no significant difference in body weight was observed between males and females of each experimental group. It is likely that treatment of juvenile tilapia with 11KT for 50 days may not be early enough or long enough to induce sex change or sex-related differences in growth rate.

We have reported earlier that when yolk-sac fry of tilapia were reared with MT-enriched feed (10 mg/kg) for 150 days, plasma IGF-I levels were significantly higher than in the control fish (Riley et al., 2002b). In another study, male tilapia treated with E_2 had significantly lower levels of plasma IGF-I and liver IGF-I mRNA. By contrast, females treated with dihydrotestosteone (DHT), a non-aromatizable androgen, showed elevated plasma IGF-I and liver IGF-I mRNA levels (Riley et al., 2002a). Immature, mixed-sex coho salmon injected with testosterone or 11KT had significantly higher plasma IGF-I levels than control fish after 1 and 2 weeks (Larsen et al., 2004). Recently, Gomez-Requeni et al. (2004) demonstrated a significant decrease in hepatic IGF-I mRNA levels in sea bream due to decreased fishmeal content of the diet. In the present study, males showed significantly higher levels of plasma IGF-I than in females, but no consistent effect of 11KT or fishmeal was observed. Plasma GH was not determined due to an insufficient volume of plasma. The present results may suggest that treatment of juvenile tilapia with 11KT (10 mg/kg fed for 50 days) may not be sufficient to induce changes in plasma IGF-I levels. As described above, long-term MT treatment in tilapia produced a significant increase in plasma IGF-I (Riley et al., 2002b). The absence of effect of 11KT in the present study may also be due to the faster clearance of this native androgen compared with that of synthetic steroids such as MT or other androgen metabolites such as DHT.

The male sex steroids, testosterone in higher vertebrates and 11KT in fish, are required for spermatogenesis, but their mode of action has remained obscure (Ijiri et al., 2008; Schulz et al., 2001). In the present study, plasma levels of 11KT were significantly higher in male tilapia than in females; no significant effect of fishmeal or 11KT was observed in females. In males, plasma 11KT levels were significantly higher in the fish fed FM diets than in those fed SM diets. It is highly likely that some components, possibly sex steroids, in FM diets may stimulate 11KT production in males. Plasma levels of 11KT were also higher in male Nile tilapia than in females (Toguyeni et al., 1997). According to Toguyeni et al. (1996), when Nile tilapia were either fasted or fed on restricted food rations for 15 days, specific growth rates were significantly related to feeding levels; however, no significant correlation was found between plasma 11KT and feeding ration. As shown in the present study, when mature females were given 11KT intraperitoneally at a dose of 1 μg/g, plasma levels of 11KT increased markedly after 4 h, decreased significantly after 8 h, and the initial level was attained after 24 h. The dose of 11KT was equivalent with the amount of 11KT in the feed (10 mg/kg), if the fish consumed 10% of body weight at one time. In fact, the fish in this study consumed only 3–4% of body weight per day. Similarly high turnover rate has also been observed for MT; when Nile tilapia fry were fed ^{3}H-MT along with 30 mg MT/kg ration for 30 days, 2.5–3.0% of whole body ^{3}H-MT residues were polar metabolites after 24 h (Curtis et al., 1991). The absence of effect of supplementary 11KT in the feed on plasma 11KT in the present study may be due to rapid turnover rate of this native steroid. In contrast, plasma levels of 11KT remained elevated up to 2 weeks post-injection in juvenile coho salmon given 0.25 or 1 μg/g 11KT (Larsen et al., 2004). In juvenile Atlantic cod, another cold-water species, injection with 5 μg/g 11KT resulted in elevated plasma 11KT that returned to control levels after 21 days (Kortner et al., 2009). Disparity in clearance of 11KT in tilapia compared with coho salmon and Atlantic cod may be attributed to differences in species or temperature-dependent kinetics.

Vitellogenin is a precursor of egg yolk protein, produced in the liver under stimulatory control of estrogens in oviparous vertebrates (Jalabert, 2005). According to Lazier et al. (1996), the treatment of mature female tilapia with high levels of MT produced a pronounced decline in serum Vg levels, along with marked reduction in serum E_2. They ascribed the reduced serum E_2 to an inhibitory feedback action of MT on GTH production at the hypothalamo-pituitary axis. In the present study, low but significant levels of Vg were found in male plasma. Plasma Vg levels were significantly lower in the fish fed SM diets compared with those fed FM diets in both males and females, clearly indicating that fishmeal contains significant estrogenic activity. Unexpectedly, plasma Vg levels were significantly higher in the males fed the FM+ diet than in the fish fed the FM−, whereas the FM+ diet significantly reduced the Vg levels in females. Nonetheless, there was no significant effect of 11KT or fishmeal on plasma E_2 levels in females; no correlation was found between plasma levels of Vg and E_2. Similarly in an earlier study, we found no effect on plasma Vg when female tilapia were treated with DHT, although the GH/IGF-I axis was significantly affected (Riley et al., 2002a). Significant amounts of fishmeal-derived sex steroids and plant-derived phytoestrogens have been detected in a variety of fish feeds; however, their potential effects on GH/IGF-I axis or Vg production are often overlooked (Inudo et al., 2004; Kato et al., 2004; Matsumoto et al., 2004). Despite less fishmeal, high levels of phytoestrogens resulted in more estrogenic activity in the carp diet (Ishibashi et al., 2002; Kobayashi et al., 2006). In a separate study, when male tilapia were fasted or fed a squid-based diet that replaced the commercial trout diet, plasma Vg was reduced to undetectable levels after 14–20 days (Davis et al., 2009). Reporter gene assays using estrogen or androgen receptor genes of a species of interest, are now commonly used to estimate estrogenic or androgenic activity in environmental samples (Iguchi et al., 2007) and estrogen and androgen receptors have been identified for tilapia (Chang et al., 1999). In order to clarify the effect of estrogenic and androgenic activities, derived from fishmeal and plant sources in the feed, on growth physiology, we are currently developing reporter gene assays using estrogen and androgen receptor genes of the tilapia.

5. Conclusion

Juvenile tilapia fed FM diets for 50 days grew significantly faster than those fed SM diets. Addition of 11KT (10 mg/kg) to the FM diet significantly augmented growth, whereas no significant effect of 11KT was observed in fish fed SM diet. In accord with the accelerated growth, specific growth rates of fish fed FM diets were significantly greater than those of fish fed SM diets, although addition of 11KT to FM or SM diet had no effect. No conspicuous effect of fishmeal or 11KT was observed in plasma levels of IGF-I, 11KT, E_2 and Vg and no sex change was observed. Plasma levels of Vg were significantly reduced in the fish fed SM diets. The absence of effect of 11KT in the fish fed SM diet may indicate that estrogenic activities or some components in SM diet may potentiate or interfere with the growth-promoting effects of 11KT.

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